Polymer-Supported Saponins: An Approach to Cholesterol Removal from Butteroil

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Digitonin and tomatine were bonded to functionalized Merrifield resin (2% divinylbenzene) at an average 0.14 mmol of saponin/g of polymer via acetal or ester linkages by condensation to aldehydic, carboxypropene, or mixed anhydride functionalized polymers. These polymer-supported saponins removed cholesterol from simple hexane solutions of the sterol or butteroil but somewhat less efficiently than polymer saponins derived from carboxy or carboxymethyl polymers. The polymers were regenerated by benzene extraction to the original cholesterol binding capacity. More importantly, acetone and ethyl acetate are equivalent to benzene as solvents for cholesterol removal.

INTRODUCTION

Association of cholesterol with degenerative heart disease has eroded the market for dairy products containing high levels of milkfat. Methods employed to remove cholesterol from butteroil include the use of complexing agents, supercritical carbon dioxide, methanol extraction (Keen et al., 1989), activated carbon (Keen, 1989), and steam stripping (*Dairy Sci. Abstr.*, 1990). The latter three procedures remove high proportions of cholesterol; however, flavor components, carotene, and other vital component triglycerides affecting thermal properties are also removed. Cholesterol removal by supercritical carbon dioxide extraction is of considerable interest because the process is flexible and the solvent is abundant, nonhazardous, and environmentally safe. Bradley (1989) reported a batch process that removed 90% of the cholesterol from butteroil, which subsequently yielded a soft spread product. The procedure was complicated, however, by the broad triglyceride distribution of butteroil. Similar results were obtained earlier by Shishikura et al. (1986) after passage of the carbon dioxide extracted oil through a column of silica gel. A subsequent paper (Kim, 1989) indicated that supercritical fluid extraction could be a continuous process. Another approach to the problem involves formation of an inclusion complex (Courregelonge and Maffrand, 1987) with β-cyclodextrin followed by aqueous extraction of the complex and excess cyclodextrin. Sample recycling afforded 80% cholesterol removal but incomplete recovery of butteroil.

The selective removal of cholesterol from butteroil (Schwartz et al., 1967) involved formation of a digitonin–cholesterol complex during passage of a hexane solution of butteroil through a column of digitonin adsorbed on Celite. At a 7/1 weight ratio of digitonin to cholesterol, complete removal of cholesterol occurred at 25 °C. Riccomini et al. (1990) described a similar method for cholesterol removal (90%) from butteroil using free saponins followed by filtration through Celite and extensive aqueous washes to eliminate residual toxic complexing agent. In neither approach was the complexing agent reusable. This would be feasible if the saponin were bonded to an insoluble substrate. Recently, Micich (1990, 1991) demonstrated that polymer-supported saponins (PSS) bound by ester and/or amide functions formed reusable substrates that complex cholesterol from hexane solutions of butteroil. Polymer-supported digitonin and tomatine were regenerated by benzene extraction, but their cholesterol binding efficiencies were less than that of free digitonin on Celite. Low binding efficiencies observed are believed to be due to distortion of the normal saponin configuration by multiple bonding and/or high saponin densities per unit of polymer surface. Identifying the saponin molecules that actively bind cholesterol, the bonding sites to the polymer, and a synthesis for this specific polymer-supported saponin would resolve the situation. It would seem that a polymer containing only actively bound saponin would bind cholesterol at a 1:1 ratio. This led to the following approaches to explore cholesterol uptake where the saponin is bound by either acetal, 1-propene-3-carboxylate, or ester functionalities. Acetal formation should restrict bonding sites on the saponin, extending the polymer carboxyl group from the benzene ring should reduce saponin distortion, and, finally, reaction with an anhydride should reduce the saponin–ester population per unit of polymer surface.

MATERIALS AND METHODS

Pyridine and dioxane were distilled from calcium hydride and stored over 4-Å molecular sieves under nitrogen. Dimethyl sulfoxide, phthaldehyde, malonic acid, trifluoroacetic anhydride, benzene, anhydrous dimethylformamide, and methylene chloride were from Aldrich Chemical, Metuchen, NJ 08840. Benzene is a carcinogen; therefore, use care in its handling. Digitonin was from ICN Biomedical Inc., Costa Mesa, CA 92626. Tomatine, cholesterol, and Merrifield resin (chloromethylated polystyrene, 2% DVB, 1.04 mequiv of chlorine/g of polymer) were from Sigma Chemical Co., St. Louis, MO 63178. Dicyclohexylcarbodiimide (DCC) was vacuum distilled and stored under nitrogen in a desiccator. Infrared spectra were obtained from KBr disks with a Perkin-Elmer 1310 microprocessor controlled infrared spectrophotometer. Visible spectra were obtained with a Beckman DU Series 70 UV–vis spectrophotometer using 1-cm matched cells. Cholesterol was determined according to the *o*-phthaldehyde–sulfuric acid colorimetric method ($\epsilon = 24\ 100\ L\ mol^{-1}\ cm^{-1}$) with a sensitivity sufficient to determine 0.1 μmol of cholesterol (Bachman et al., 1976). Variations in absorbance for calibration curves from 0 to 80 μg of cholesterol indicate a relative error for cholesterol concentration of ±8%. Butteroil was prepared from locally purchased sweet unsalted butter. The butter was liquefied and centrifuged, and the oil was separated from the aqueous phase. The oil was vacuum filtered through double filter paper, yielding a clear filtrate of anhydrous butteroil which assayed at 0.29 wt % cholesterol. The sample was stored under nitrogen at −20 °C.

Aldehydic Polymer (PS-I, Figure 1). This is a variation of a method described by Frechet and Schuerch (1971). Merrifield resin (30 g; 1.04 mequiv of Cl/g of polymer) was oxidized with 200 mL of dimethyl sulfoxide containing 13 g of sodium bicarbonate at 155–160 °C for 8 h. The resin was quantitatively transferred to a sintered glass funnel with dimethyl sulfoxide and washed successively with the following warm solvents (4 × 50 mL): dimethyl sulfoxide, water, water/dioxane 2:1, 1:1, and 1:2, dioxane, dioxane/ethanol 1:1, and methanol. The initial wash liquid was allowed to drain by gravity (~5 min), with the remaining solvent removed by suction filtration. The off-white polymer after vacuum drying weighed 30 g and showed a carbonyl band at 1688 cm^{-1} and the absence of the carbon chlorine band at 1258 cm^{-1}. The oxime derived from the polymer contained 1.00% nitrogen, corresponding to 0.73 mmol of CHO/g of polymer.

1-Propene-3-carboxy Polymer (PS-II, Figure 1). This product was derived from aldehydic polymer PS-I according to the method of Frechet and Schuerch (1971). The polymer contained 0.62 mequiv of CO_2H/g of polymer and exhibited a broad carbonyl band at 1700 cm^{-1} and an unsaturation band at 1622 cm^{-1}.

Carboxymethyl Polymer. Carboxymethylpolystyrene (CMPS), (2% divinylbenzene) containing 1.00 mmol of CO_2H/g of polymer, derived from Merrifield resin, was prepared by the method of Kusama and Hayatsu (1970).

Saponin Condensation with Aldehydic Polymer (PS-I). *Acetal Formation with Digitonin.* This is a modification of a procedure by Frechet and Pellé (1975). Aldehydic polymer PS-I (2 g) (1.66 mmol of CHO) was treated under dry nitrogen with 65 mL of anhydrous dioxane, 50 mg (0.26 mmol) of toluenesulfonic acid monohydrate, and 1 g (0.81 mmol) of dry digitonin. The flask was equipped with a Soxhlet cup containing ~30 mL of 3-Å molecular sieves and a drying tube. A Kimax distillation head, capacity 15 mL, cat. no. 29002, could also be used containing ~15 mL of 3-Å molecular sieves. The mixture was stirred at reflux for 48 h. The dark reaction mixture was treated with 2 mL of pyridine and quantitatively transferred to a sintered glass funnel. The polymer was washed with the following warm solvents (3 × 10 mL), which were allowed to drain gravimetrically for 5 min and then removed by suction: dioxane, dioxane/H_2O 2:1, 1:1, and 1:2, water, ethanol, and methanol. The polymer was vacuum dried at <0.1 mmHg to constant weight of 2.39 g. The polymer (PSS-ID) contained 0.16 mmol of digitonin/g of polymer as calculated from weight gain and elemental analysis. It exhibited a strong OH band at 3440 cm^{-1} and a weak shoulder at 1688 cm^{-1}.

Acetal Formation with Tomatine. Aldehydic polymer PS-I (2.0 g) (1.66 mmol) was dispersed in 35 mL of anhydrous dimethylformamide and 20 mL of anhydrous benzene to which was added under dry nitrogen 80 mg (0.42 mmol) of toluenesulfonic acid monohydrate and 0.85 g (0.81 mmol) of dry tomatine. The flask was equipped with a Kimax distilling trap containing ~15 mL of 3-Å molecular sieves and a drying tube. The mixture was stirred at reflux for 48 h, cooled, treated with 2 mL of pyridine, and allowed to stand for several hours. The mixture was filtered and washed successively with the following solvents (3 × 10 mL): dimethylformamide, water, dioxane, dioxane/benzene 2:1, 1:1, and 1:2, methylene chloride, and ethanol. After vacuum drying to constant weight, the polymer (PSS-IT) weighed 2.42 g and contained 0.2 mmol of tomatine/g of polymer as determined by microanalysis. The IR spectrum of PSS-IT showed a strong OH band at 3440 cm^{-1} and essential disappearance of the 1688-cm^{-1} carbonyl band.

Condensation of 1-Propene-3-carboxypolystyrene (PS-II) to Digitonin. Digitonin, 0.62 g (0.50 mmol), was dried with 2.00 g (1.24 mmol) of PS-II at 105 °C and <0.1 mmHg for 1.5 h. To these components under nitrogen was added 13 mL of anhydrous pyridine and 0.51 g (2.50 mmol) of dicyclohexylcarbodiimide. The flask was sealed with a drying tube and stirred for 45 min at room temperature and then for 24 h at 5 °C. The straw-colored reaction mixture was treated with 15 mL of water and stirred overnight at 25 °C. The mixture was quanitiatively transferred to a sintered glass funnel with water and filtered. The polymer residue was washed with the following warm solvents (3 × 10 mL): pyridine, pyridine/benzene 1:1, benzene, benzene/ethanol 2:1, 1:1, and 1:2, ethanol, and methanol. Vacuum drying the residue at 70 °C and <0.1 mmHg for 1 h gave 2.13 g of polymer containing 0.06 mmol of digitonin/g of polymer (PSS-IID) from microanalysis. The product showed a broad OH band at 3440 cm^{-1}, with shoulders at 1715 and 1680 cm^{-1} in the carbonyl region.

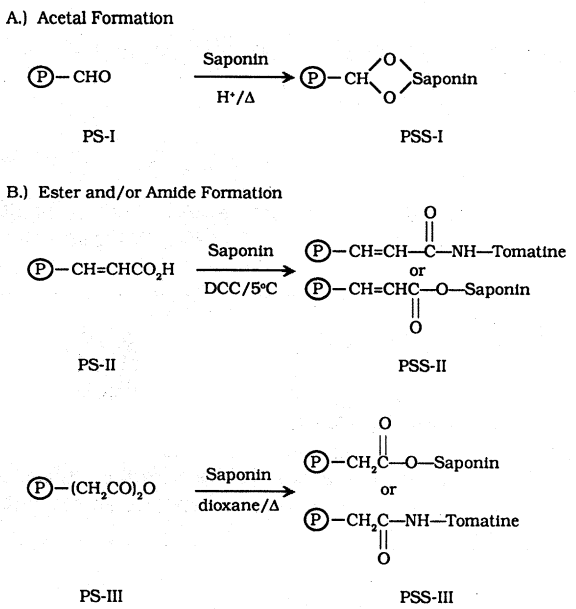

Figure 1. Synthesis of polymer-supported saponins. Addition of aldehydic polymer PS-I to digitonin or tomatine occurs at the 4,6-hydroxy groups of the oligosaccharide. Formation of polymer saponins II and III will bind digitonin by an ester group and tomatine by both ester and amide linkages.

Tomatine was added to PS-II using the same conditions except that the reaction time was extended to 80 h to yield 2.14 g of polymer containing 0.07 mmol of tomatine/g of polymer (PSS-IIT) from microanalysis. The product gave the same IR peaks as observed with PSS-IID along with a possible amide carbonyl shoulder at 1650 cm^{-1}.

Condensation of Carboxymethylpolystyrene Anhydride (PS-III) with Digitonin. Polymeric anhydride PS-III was prepared from CMPS using the procedure of Crowley et al. (1973). To 2.00 g of dry PS-III was added a solution of 0.75 g (0.61 mmol) of digitonin dissolved in 15 mL of dry dioxane under nitrogen. The stirred mixture was refluxed for 18 h, cooled, and quantitatively transferred to a sintered glass funnel with dioxane. The polymer-supported saponin, PSS-IIID, was washed with the following warm solvents (3 × 10 mL): dioxane, dioxane/H_2O 2:1, 1:1, and 1:2, water, ethanol, and methanol. After drying, the tan PSS-IIID weighed 2.16 g, which contained 0.07 mmol of digitonin/g of polymer by microanalysis. The IR spectrum of PSS-IIID showed a broad carbonyl band at 1710 cm^{-1} with a shoulder at 1730 cm^{-1} and an OH band at 3440 cm^{-1}.

The same procedure was used for the addition of tomatine to a 2-g sample of PS-III except that reflux time was extended to 48 h. The dried polymer product PSS-IIIT weighed 2.18 g, indicating the presence of 0.09 mmol of tomatine/g of polymer by microanalysis. The IR spectrum was the same as found with PSS-IIID with an additional amide carbonyl band at 1670 cm^{-1}.

Treatment of PSS with Cholesterol or Butteroil in Hexane. This procedure was previously described for digitonized polymers (Micich, 1990) and is directly applicable to the PSS shown in Figure 1.

Regeneration of PSS. A polymer sample, 0.40 g, treated with cholesterol in hexane was centrifuged and the hexane supernatant discarded. The residue was treated with 5 mL of benzene, stirred vigorously for 10 min, and centrifuged. The supernatant was carefully decanted into a 25-mL volumetric flask and diluted with hexane. The polymer residue was treated 4 times again with 5 mL of benzene, and after each centrifugation, the benzene extract was decanted into a screw-capped vial and used as obtained. A 1-mL aliquot of each extract was pipetted into a 10-mL volumetric flask and solvent removed on the steam-bath under nitrogen. The color reaction (Micich, 1990) was

Table I. Cholesterol Uptake by Polymer-Supported Saponins (PSS)[a] I–III after 48 h

sample	PSS[b]	mmol of saponin/g of PS[c]	mg of cholesterol uptake/g of PSS	wt of saponin/wt of cholesterol
1	ID	0.16	2.3	87
2	IT	0.20	2.9	72
3	IT	0.28	1.8	161
4	IT	0.18	0.9	211
5	IID	0.06	1.8	39
6	IIT	0.07	0.9	78
7	IIID	0.07	1.6	56
8	IIIT	0.09	1.1	82
CMPS[d]		0	0.6	0

[a] PSS-I contained 0.73 mmol of CHO/g of polymer, PSS-II contained 0.62 mmol of CO_2H/g of polymer, and PSS-III contained 1.00 mmol of CO_2H/g of polymer. [b] D, digitonin; T, tomatine; data are based on 0.40-g PSS samples. [c] PS, polymer support. [d] CMPS, carboxymethylpolystyrene, which absorbs 0.6 mg of cholesterol/g and is the control for each series.

performed on each extract and cholesterol content determined from a calibration curve (0–80 μg of cholesterol).

A check on cholesterol removal from the extracted polymer was made by quantitatively transferring the polymer to a micro-Büchner funnel and drying to constant weight. Twenty-five milligrams of PSS was weighed into a 10-mL volumetric flask. A blank sample containing 25 mg of control polymer (without saponin) was run simultaneously. The color reaction was performed as described (Micich, 1990). After the necessary time for color development, the samples were filtered and absorbance was measured at 552.5 nm. Cholesterol content was determined from the calibration curve.

RESULTS AND DISCUSSION

Reactions used to bond digitonin and tomatine to polymer supports are shown in Figure 1, and the products are referred to as polymer-supported saponins PSS-I, -II, and -III. Chloromethylated Merrifield resin was conveniently oxidized with mildly alkaline dimethyl sulfoxide to the aldehydic polymer PS-I after 8 h at 160 °C. Acetalization of PS-I with digitonin to give PSS-ID occurred using catalytic amounts of toluenesulfonic acid in anhydrous dioxane. No addition of tomatine to PS-I occurred using dioxane under these conditions; however, the reaction to form PSS-IT proceeded using a 2:1 or 1:1 mixture of dimethylformamide and benzene.

Carboxypropenepolystyrene PS-II formed readily by reacting aldehydic polymer with malonic acid in refluxing pyridine. Digitonin reacted with PS-II using DCC at twice the concentration of PS-II to yield PSS-IID. Reaction of PS-II with tomatine had to be carried out for an extended period of time under the same conditions to obtain the same amount of saponin bonded to PS-II.

Repeated treatment of carboxymethylpolystyrene (CMPS) with trifluoroacetic anhydride affords both mixed and intraresin anhydride (PS-III) (Crowley et at., 1973), with an excess of the latter. Decomposition of the mixed anhydride with aqueous dioxane and reaction of the remaining intraresin anhydride with a saponin should lead to polymer saponin III with better cholesterol scavenging ability than that observed with the same products obtained via the polymer acid chloride or carboxylic acid (Micich, 1990, 1991). Unfortunately, no reaction was observed between the intraresin polymer anhydride and digitonin after refluxing in dioxane for 24 h. However, treatment of polymer samples of mixed and intraresin anhydride with digitonin or tomatine gave PSS-III digitonin or tomatine adducts.

Cholesterol uptake from hexane solution for the three types of PSS is shown in Table I. Saponin concentrations of 0.06–0.28 mmol/g of polymer, representing utilization of 10–30% of the functional group concentrations in the precursor polymers, bind cholesterol at 0.9–2.9 mg/g of polymer. The PSS with the highest saponin concentrations, samples 3 and 4 (column 3), show the lowest cholesterol uptake values, suggesting decreasing efficiency with increasing saponin concentration as reflected in column 5. However saponin to cholesterol ratios (column 5) showed comparable activity for the remaining PSS listed in column 2. These polymer saponins do not compare to free digitonin deposited on Celite, which binds cholesterol at a 7/1 weight ratio (Schwartz et al., 1967), but are of the same order of magnitude as found earlier (Micich, 1990, 1991) with CMPS. Polymer sample identifications shown in Table I refer to the same samples described in Tables II–IV. The actual cholesterol content of samples shown in Tables I–IV is the sum of the cholesterol bound to the polystyrene support and that bound to the saponins.

The effectiveness of benzene extraction in removing cholesterol from the various PSS cholesterol adducts is shown in Table II. After the uptake determinations were completed, the samples were extracted with benzene and recovered cholesterol was determined. Initial cholesterol uptakes from hexane for the polymer saponins are shown in column A of Table II. Two recycles are shown in columns B and C. Cholesterol uptake was determined after at least 96 h of reaction time. After each series, the polymer samples (0.40 g) were stripped of cholesterol by benzene extraction and any sample loss was replaced by original polymer saponin. Thus, column A shows cholesterol uptake by the original sample of PSS followed by benzene extraction, while columns B and C show uptake by recycled PSS after benzene extraction. The data in Table II show little difference in initial cholesterol uptake between PSS derived from digitonin or tomatine; the samples are reusable after two regenerations with minimal loss in cholesterol uptake capacity. Finally, samples 3 and 4 (Table II) containing high concentrations of saponin are the least efficient. Good agreement was found between cholesterol bound to the PSS and that recovered by benzene extraction. The removal of cholesterol from these PSS cholesterol adducts by extraction indicates that the complex formed with these polymer saponins is similar to those polymer saponins previously studied (Micich, 1990, 1991).

Cholesterol uptake from hexane solutions of butteroil for representative PSS and regeneration of the PSS as just described are shown in Table III. For each sample, cholesterol content was determined after 24, ≥96, and ≥120 h. Maximum uptake occurred after at least 96 h. Comparison of these data with those in Table II shows that uptake of cholesterol from butteroil is 10–40% less efficient than from simple hexane solutions for samples 1, 2, and 5. After one recycle, sample 7 appears to show the same cholesterol uptake (columns B and C) from butteroil or hexane solutions of the sterol. This may well be an anomaly because past studies (Micich, 1990, 1991) have shown that butteroil triglycerides interfere significantly with cholesterol uptake. After one recycle, samples 1 and 2 show a noticeable increase in cholesterol uptake. No such changes occur with samples 5 and 7 after two regenerations.

The effect of solvents on cholesterol uptake by two PSS-I samples after complexing times of at least 48 h showed that both hexane, a nonpolar solvent, and methanol, a polar protic solvent, permitted comparable binding of cholesterol to the PSS (data not shown). This result contrasts with previous work which showed that hexane

Table II. Cholesterol Removal from Polymer-Supported Saponin (PSS)[a] Cholesterol Adducts I–III by Benzene Extraction

			mg of cholesterol/g of PSS for series					
			A		B		C	
PSS adduct	PSS[b]	mmol of saponin/g of PS[c]	uptake	recovered	uptake	recovered	uptake	recovered
1	ID	0.16	2.9	3.0	3.3	2.5	3.4	3.0
2	IT	0.20	3.0	3.9	4.1	4.0	3.9	3.8
3	IT	0.28	0.9		1.8		1.5	
4	IT	0.18	0.2	0.7	0.7	0.8	0.9	1.0
5	IID	0.06	1.6	1.5	1.4	1.4	1.5	1.2
6	IIT	0.07	0.8	1.0	0.8	1.0	0.8	0.6
7	IIID	0.07	1.6	0.8	0.8	1.0	0.9	0.7
8	IIIT	0.09	1.0	0.5	1.2	0.5	1.0	0.5
CMPS[d]		0	0.8	0.9	1.2	1.5	0.8	1.0

[a] PSS-I contained 0.73 mmol of CHO/g of polymer, PSS-II contained 0.62 mmol of CO_2H/g of polymer, and PSS-III contained 1.00 mmol of CO_2H/g of polymer. [b] D, digitonin; T, tomatine; data are based on 0.4 g of PSS. [c] PS, polymer support. [d] CMPS, carboxymethylpolystyrene, which absorbs 0.6 g of cholesterol/g and is the control for each series.

Table III. Cholesterol Uptake from Butteroil by Regenerated Polymer-Supported Saponins (PSS)[a] I–III

			mg of cholesterol uptake/g of PSS for series								
			A			B			C		
sample	PSS[b]	mmol of saponin/g of PS[c]	24 h	108 h	128 h	24 h	96 h	120 h	24 h	96 h	120 h
1	ID	0.16	0.3	0.3	0.7	0.3	1.1	1.3	0.5	1.3	1.2
2	IT	0.20	0.5	0.7	0.3	0.6	1.3	1.5	0.4	1.7	1.6
5	IID	0.06	0.5	0.8	0.4	0.4	0.6	0.4	0.7	0.4	0.9
7	IIID	0.07	0.3	0.7	0.5	0.3	0.8	1.0	0	0.7	0.7
CMPS[d]		0	0.7	0.8	0.9	0.3	0.1	0.2	0.2	0.3	0.3

[a] PSS-I contained 0.73 mmol of CHO/g of polymer, PSS-II contained 0.62 mmol of CO_2H/g of polymer, and PSS-III contained 1.00 mmol of CO_2H/g of polymer. [b] D, digitonin; T, tomatine; data are based on 0.4 g of PSS. [c] PS, polymer support. [d] CMPS, carboxymethylpolystyrene, which absorbs 0.6 g of cholesterol/g and is the control for each series.

Table IV. Cholesterol Removal from Polymer-Supported Saponin (PSS)[a] I by Aliphatic Solvents

PSS adduct	mmol of saponin/g of PS[b]	solvent	mg of cholesterol/g of polymer present	mg of cholesterol/g of polymer extracted[c]
2	0.20 T	methanol	3.5	4.3 + 0.7
2	0.20 T	ethanol	1.1	2.0 + 0.1
2	0.20 T	acetone	2.4	3.3 + 0
1	0.16 D	ethyl acetate	0.9	1.7 + 0

[a] PSS-I contained 0.73 mmol of CHO/g of polymer. [b] PS, polymer support: D, digitonin; T, tomatine. [c] Cholesterol removed by the solvent shown plus that removed by an equivalent benzene extraction.

was the preferred solvent for the binding of cholesterol to PSS (Micich, 1990). Decreasing uptake by PSS-I was observed with 95% ethanol or ethanol and essentially zero uptake by aqueous ethyl acetate and 95% isobutyl alcohol. Aqueous solvents were evaluated because of the possibility that water is essential for rapid cholesterol uptake (Schwartz et al., 1967), a conclusion not verified by our experimental results. From the foregoing, we have concluded that hexane is still the preferred solvent for binding of cholesterol to polymer-supported saponins.

An evaluation of solvents other than benzene to remove cholesterol from polymer saponin I complexes is shown in Table IV. Using the same extraction procedure as previously described with benzene, it is seen that ethyl acetate and acetone remove all cholesterol, while methanol and ethanol effect only partial removal from the PSS. After each solvent extraction, the polymer samples were extracted with benzene to check for residual cholesterol. These data comprise the second set of values under the "extracted" column.

As the data show, acetone and ethyl acetate removed all complexed cholesterol from the PSS. This is important because use of the latter solvents is much preferred over benzene and thus enhances the potential of the polymer-supported saponin approach as a means of removing cholesterol from foods that contain this sterol.

The data developed in this study indicate the following: Polymer saponins I–III bind cholesterol equally, with the more active sample containing the lowest saponin content. Their cholesterol binding ability is comparable to that of previously studied polymer-supported saponins (Micich, 1990, 1991), which differ in chemical binding or saponin structure. Likewise, the PSS can be regenerated to their original cholesterol binding capacity by a simple benzene extraction, thus making the PSS reusable. Cholesterol uptake in butteroil is lower than in simple hexane solutions of the sterol. Methanol appears to be as effective as hexane in promoting cholesterol uptake, but, most importantly, acetone and ethyl acetate are comparable to benzene in removing cholesterol from polymer-supported saponins.

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